

PCT-patent application PCT/EP2004/003921  
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### CLAIMS

1. A method of producing single-stranded nucleic acid molecules from oligo- or polynucleotides wherein each of said oligo- or polynucleotides has a predefined 5' or 3' terminus, comprising the steps of
  - (a) annealing an adaptor oligonucleotide simultaneously or step by step to
    - (aa) a first oligo- or polynucleotide; and
    - (ab) a second oligo- or polynucleotidewherein the 5'-terminus of said adaptor oligonucleotide is complementary in sequence to the 5' terminus of said first oligo- or polynucleotide and the 3'-terminus of said adaptor molecule is complementary in sequence to the 3' terminus of said second oligo- or polynucleotide; and optionally
  - (a') simultaneously with or subsequently to step (a) annealing at least one further adaptor oligonucleotide to free termini of said first or second oligonucleotides and to free termini of further oligo- or polynucleotides;
  - (b) optionally filling in gaps between the neighbouring ends of said oligo- or polynucleotides;
  - (c) ligating said oligo- or polynucleotides; and
  - (d) removing said at least one adaptor oligonucleotide,wherein said single-stranded nucleic acid molecules represent a collection of nucleic acid molecules and wherein either said first or said second oligo- or polynucleotide is invariable in sequence between all members of said collection of nucleic acid molecules.
2. The method of claim 1 wherein the complementarity in sequence is at least four nucleotides.
3. The method of claim 1 or 2 wherein annealing and ligation are simultaneously performed.

4. The method of any one of claims 1 to 3 wherein the adaptor oligonucleotide(s) in step (a) and/or (a') is/are provided in molar excess over the first or second or further oligo- or polynucleotides.
5. The method of any one of claims 1 to 4 wherein said first or said second oligo- or polynucleotide which is not invariable is variable in sequence between different members of said collection of nucleic acid molecules.
6. The method of any one of claims 1 to 5 wherein the further oligo- or polynucleotides are variable in sequence between different members of said collection of nucleic acid molecules.
7. The method of any one of claims 1 to 6 wherein the oligo- or polynucleotides representing said variable sequences are provided in molar excess over the nucleic acid molecule representing said invariable sequences.
8. The method of any one of claims 1 to 7 wherein the 5' or 3' termini of said oligo- or polynucleotides representing said variable sequences which anneal to said 5' or 3' termini of said adaptor oligonucleotide are invariable between different members of said oligo- or polynucleotides representing said variable sequences.
9. The method of any one of claims 1 to 8 where ligation is effected with T4/DNA ligase.
10. The method of any one of claims 1 to 9 wherein the ligation reaction is carried out in the presence of at least 5% polyethylene glycol.
11. The method of claim 7 wherein the ligation reaction is carried out in the presence of about 15% polyethylene glycol.
12. The method of claim 10 or 11 wherein said polyethylene glycol is polyethylene glycol 6000.
13. The method of any one of claims 1 to 10 wherein about 1 unit of T4 DNA ligase is reacted in step (c) with about 4 pmol of termini of the oligo- or polynucleotides annealed to said adaptor molecule(s).

14. The method of any one of claims 1 to 11 further comprising the step of purifying said single-stranded nucleic acid molecules.
15. The method of claim 14 wherein purification includes PAGE electrophoresis, HPLC or chromatography.
16. The method of any one of claims 1 to 15 further comprising modifying at least one of said oligo- or polynucleotides.
17. The method of any one of claims 1 to 15 wherein at least one of said oligo- or polynucleotides is modified.
18. The method of claim 16 or 17 wherein the modification is a ribonucleotide, a spacer or a nucleotide comprising a detectable label.
19. The method of any one of claims 16 to 18 wherein said oligo- or polynucleotides representing the invariable sequence are modified.
20. The method of any one of claims 1 to 19 further comprising employing members of said collection of nucleic acid molecules in the determination of SNPs in vitro.
21. The method of any one of claims 1 to 19 further comprising employing members of said collection of nucleic acid molecules in ligase-independent cloning or two-step PCR.